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GENERATION SAMPLING AND ANALYSIS FOR LOW-LEVEL GB (SARIN) VAPOR FOR INHALATION TOXICOLOGY STUDIES

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14. ABSTRACT This study tested and optimized various methodologies to generate, sample, and characterize sarin (GB) test atmospheres in an inhalation chamber, particularly at low-vapor levels. A syringe drive/spray atomization system produced GB vapor at lethal concentrations of 1-44 mg/m ³ . A saturator cell was used to generate GB vapor at sub-lethal concentrations from 1 mg/m ³ down to very low GB levels approaching the TLV-TWA of 0.0001 mg/m ³ . Both generation techniques demonstrated the ability to produce stable vapor concentrations over extended exposure periods. This capability was important to determine sub-lethal nerve agent effects for inhalation toxicology studies. In addition, the techniques employed for producing and maintaining low-level GB vapor would lay the foundation for testing other chemical warfare agents, such as GF or VX.					
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PREFACE

The work described in this report was authorized under Project No. 201400, Low Level Toxicology. This work was started in February 2000 and completed in June 2002. The experimental data are recorded in laboratory notebooks 99-0102, 00-0101, 01-0083, and 02-0059. The storage location for all the raw data and final report is in the Toxicology Archives, Building E3150, Aberdeen Proving Ground, MD.

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GENERATION SAMPLING AND ANALYSIS FOR LOW-LEVEL GB (SARIN) VAPOR FOR INHALATION TOXICOLOGY STUDIES

1. INTRODUCTION

Numerous generation, sampling, and analytical techniques have been reported for conducting vapor exposures in an inhalation chamber.¹ Vapor generation systems have usually consisted of permeation devices, diffusion cells, liquid injection with heating, and/or direct evaporation of a liquid.^{2,3} Vapor collection and analysis techniques have typically used solvent bubblers, solid sorbent tubes, and/or gas sample loops followed by gas chromatographic (GC) analysis.

In the past, many of these techniques have been used for inhalation toxicity studies of chemical warfare (CW) agents. These studies have primarily focused on lethal effects that required high vapor concentrations for short-term exposures. However, concerns about worker health and safety, Gulf War syndrome, and physical protective measures (i.e., protective masks, clothing, detectors) have prompted a renewed emphasis on the effects of low-level agent exposures.⁴

This study tested various vapor generation, sampling, and analysis systems to assess different levels of toxicity (low-to high-vapor concentrations) in an inhalation chamber. In particular, a combination of these three systems was needed to generate stable low sarin (GB) vapor concentrations approaching the TLV-TWA of 0.0001 mg/m³.⁵ A good starting point for developing this system was to use the nerve agent GB. Sarin has a higher volatility compared to the other agents; subsequently, this system would help lay the foundation for testing less volatile agents such as GF or VX.

2. MATERIALS AND METHODS

2.1 Chemicals.

The chemical agent used for these studies was supplied in 5-mL sealed ampules (with nitrogen) under lot # GB-U-6184-CTF-N. This particular lot was part of the chemical agent standard analytical reagent material (CASARM) grade agent with a certified purity of 98.7 ± 1.9 wt % GB as determined by acid-base titration. Verification of the CASARM grade GB was performed by quantitative NMR³¹P, using triethylphosphate (99.9% purity, Aldrich Chemicals (Milwaukee, WI) as the internal standard.⁶ The NMR³¹P analysis showed 97.2 wt % GB with impurities of 0.34 wt % o, o'-diisopropyl methylphosphonate (DIMP), 0.33 wt % Isopropyl methylphosphonic acid (IMPA), and 0.55% methylphosphonofluoridic acid (Fluor Acid).

Ampules were opened as needed to prepare external standards or to be used as neat agent for vapor dissemination. All external standards for GB vapor quantitation were prepared on a daily basis. Hexane solvent (GC grade) was used for standard preparation.

Chemical and Physical Properties.

Among the traditional nerve agents (G-agents), GB has the highest volatility and vapor pressure, and hence poses the greatest inhalation hazard. Pertinent physical and chemical data for vapor exposures of GB are listed in Table 1.

Table 1. Physical and Chemical Data for GB⁷

Chemical Name	Isopropyl methyl phosphono fluoridate
Molecular Formula	C ₄ H ₁₀ FO ₂ P
CAS Number	107-44-8
Vapor Density Relative to Air	4.8
Volatility @ 25 °C	2.2 x 10 ⁴ mg/m ³
Vapor Pressure @ 25 °C	2.9 mm Hg
Boiling Point	158 °C

2.2 GB Test Atmosphere System, Overview.

The vaporization system (syringe drive or saturator cell) was contained in a generator box, which in turn was connected to the inlet of a dynamic flow inhalation chamber (Figure 1). High vapor concentrations in the chamber (2-44 mg/m³ GB) were generated with a syringe drive/spray atomization system. Low vapor concentrations (0.0002-0.10 mg/m³ GB) were generated using a saturator cell. The GB vapor was monitored in the chamber with sorbent tube sampling followed by thermal desorption and GC analysis. A phosphorus analyzer also continuously monitored GB vapor at levels exceeding 0.005 mg/m³.

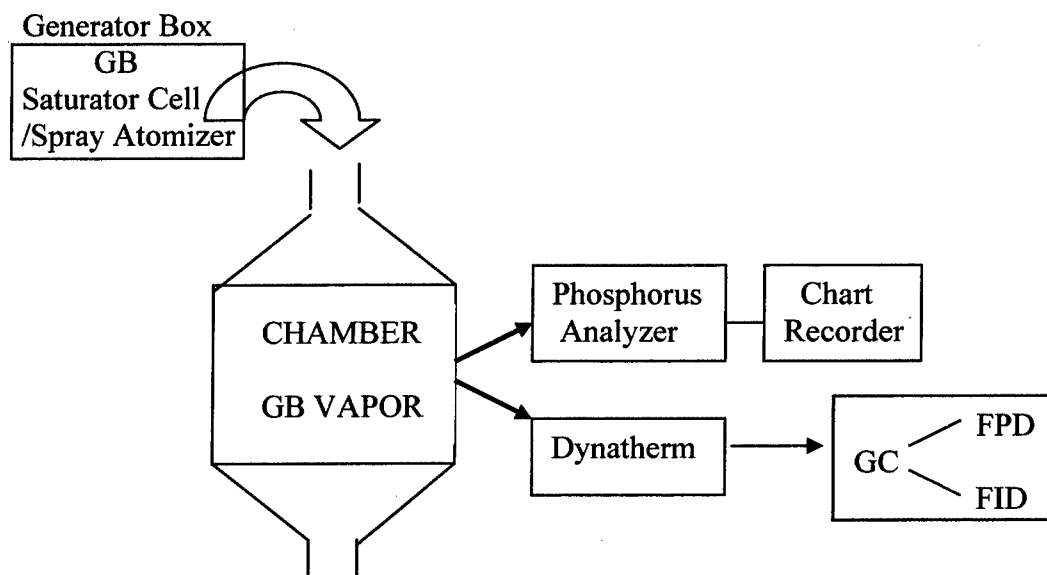


Figure 1. GB Inhalation Chamber and Monitoring Systems

2.3 Vapor Generation Systems.

2.3.1 Syringe Drive/Spray Atomization System.

Prior to chamber operation, the liquid GB was drawn into a gas-tight syringe (Hamilton, Reno, NV), transported to the generator box, and then mounted onto a variable rate syringe drive (Model 22, Harvard Apparatus Inc., South Natick, MA). Once activated, the syringe drive delivered a constant flowrate of GB (microliter/minute) through a flexible plastic line (~ 8") into a spray atomization system (Spray Atomization Nozzle 1/4 J SS, Spraying Systems Company, Wheaton IL) (Figure 2). The atomizer was modified by inserting a syringe needle (SS 25 gauge 3") into the top of the sprayer to decrease the orifice size. As liquid GB entered through the top of the atomizer, compressed air (30-40 psi) entered through the side to atomize the liquid into fine droplets. Due to the volatility of GB, these droplets quickly evaporated into GB vapor, which were then drawn down through the chamber.

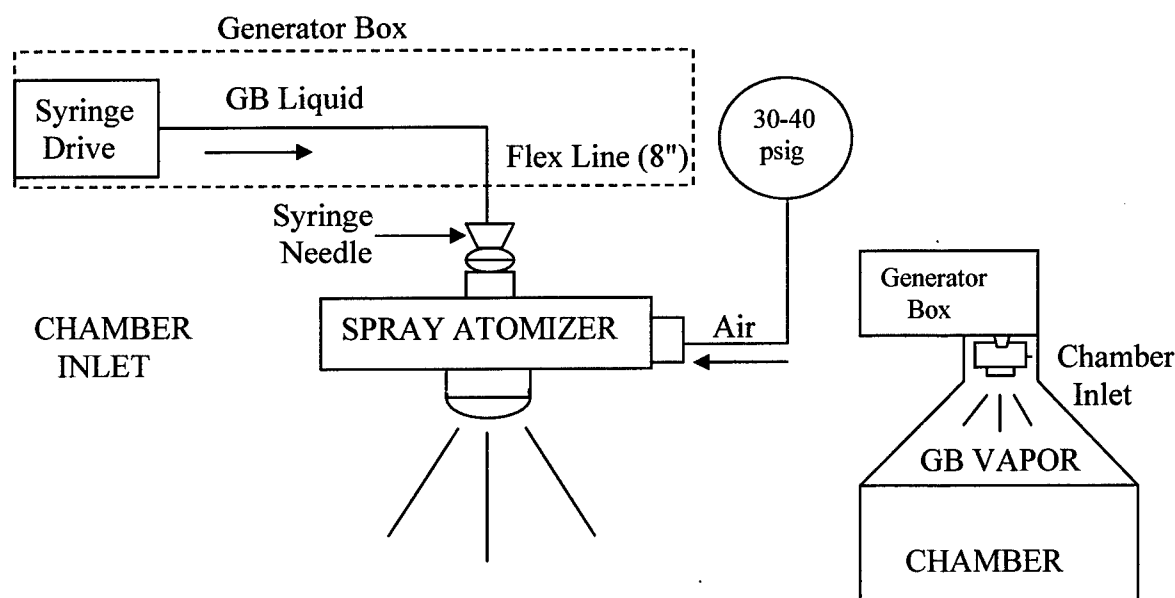


Figure 2. Spray Atomization System

2.3.2 Saturator Cell.

Saturated GB vapor streams were generated by flowing nitrogen carrier gas through a glass vessel (multi-pass saturator cell) containing liquid GB (Figure 3). The saturator cell consisted of a 100-mm long, 25-mm outside diameter (o.d.) cylindrical glass tube with two (inlet, outlet) vertical 7-mm o.d. tubes connected at each end. The main body of the saturator cell contained a hollow ceramic cylinder that served to increase the contact area between the liquid GB and the nitrogen. The saturator cell was fabricated to allow nitrogen to make three passes along the surface of the wetted ceramic cylinder (alundum® fused alumina, Norton Company, Colorado Springs, CO) before exiting the outlet arm of the glass cell. The cell body was also immersed in a constant temperature bath so that a combination of nitrogen flow and

temperature could regulate the amount of GB vapor going into the inhalation chamber. The GB vapor concentrations from the saturator cell at various temperatures and carrier flows in an inhalation chamber are illustrated in Appendix A.

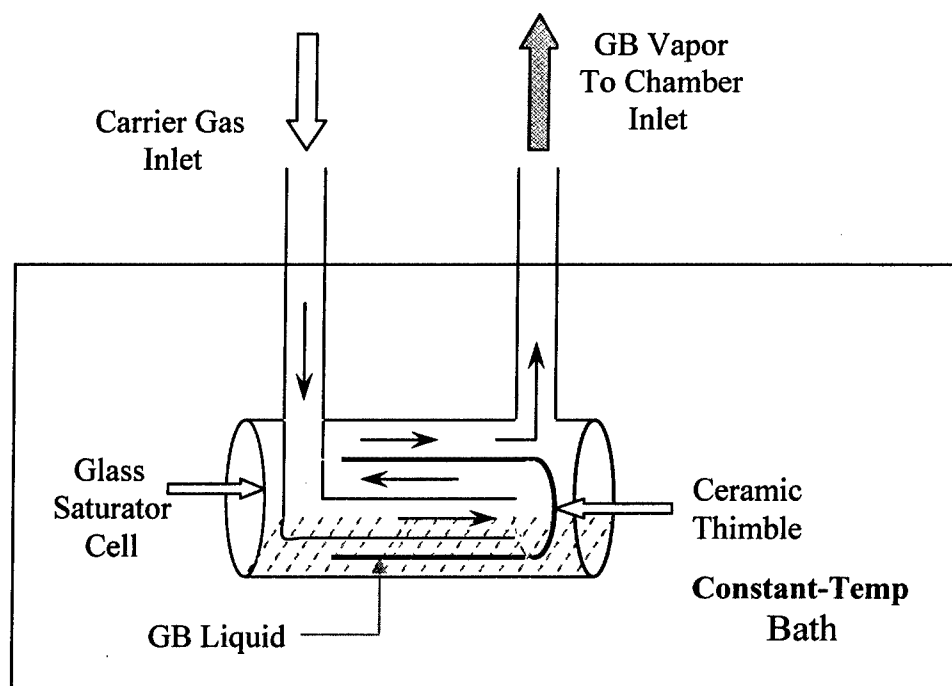


Figure 3. GB Vapor Generation via Saturator Cell

Typically, the saturator cell was loaded with 2-3 mL of liquid GB (CASARM grade). Immediately after loading, a low nitrogen flowrate (1-2 mL/min) continuously flowed through the cell to maintain the integrity of the liquid GB. This allowed the saturator cell to be used as a generation source for approximately 1-2 weeks.

2.4 Inhalation Chamber.

The GB vapor was monitored in a 750 L dynamic airflow inhalation chamber. The Rochester style chamber was constructed of stainless steel with Plexiglas windows on each of its six sides. The interior of the exposure chamber was maintained under negative pressure (0.25" H₂O), which was monitored with a calibrated magnehelix (Dwyer, Michigan City, IN). A thermoanemometer (Model 8565, Alnor, Skokie, IL) was used to monitor chamber airflow at the chamber outlet.

2.5 Sampling System.

2.5.1 Sorbent Tube System.

The automated solid sorbent tube sampling system consisted of the following four parts: a heated sample transfer line; heated external switching valve; thermal desorption unit

(Dynatherm); and a gas chromatograph (Figure 4). A stainless steel sample line (1/16" o.d. x 0.004" i.d. x 6' length) extended from the middle of the chamber to an external sample valve. The sample line was commercially treated with a silica coating (Silicasteel® Restek, Bellefonte, PA) and covered with a heated (60 °C) sample transfer line (CMS, Birmingham, AL). The combination line coating and heating minimized GB absorption onto sample surfaces. From the transfer line, the sample entered a heated (125 °C) 6-port gas-switching valve (UWP, Valco Instruments, Houston, TX). In the by-pass mode, GB vapor from the chamber continuously purged through the sample line and out to a charcoal filter. In the sample mode, the gas sample valve redirected GB vapors from the sample line to a Tenax TA/Haysep sorbent tube (60-80 mesh) located in the Dynatherm (ACEM-900, CDS, Oxford, PA). Temperature and flow programming within the Dynatherm desorbed GB from the sorbent tube directly onto the GC column (RTX-5, 30m, 0.32mm i.d., 1 mm thickness), which was then followed by either flame ionization detection (FID) or flame photometric detection (FPD-phosphorus mode). Instrument parameters for the GC and Dynatherm are listed in Appendix B. Valving positions for the switching valve and Dynatherm during various stages of sampling and transfer are illustrated in Appendix C.

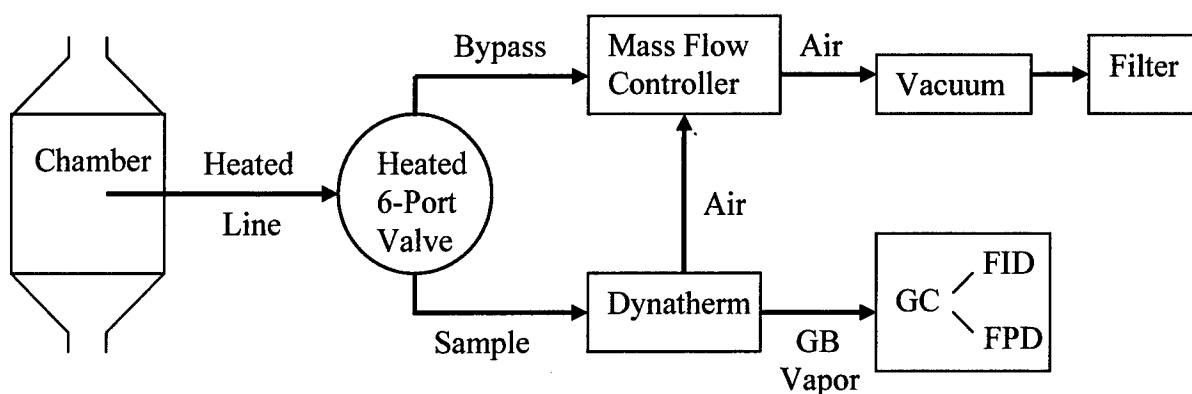


Figure 4. Automated Sorbent Sampling of GB Vapor from the Chamber

Sample flowrates for the sorbent tube systems were controlled with calibrated mass flow controllers (Matheson Gas Products, Montgomeryville, PA). Typical flowrates were 0-100 sccm for the sorbent tubes. Flowrates were verified before and after sampling by temporarily connecting a calibrated flowmeter ("DryCal", Bios International, Pompton Plains, NJ) in-line to the sample stream.

The solid sorbent tube sampling system was calibrated by direct injection of external standards (GB/hexane-micrograms/milliliter) into the heated sample line of the Dynatherm. In this way, injected GB standards were put through the same sampling and analysis stream as the chamber samples. A linear regression fit ($r^2 = 0.999$) of the standard data was used to compute the GB concentration of each chamber sample.

2.5.2 Phosphorus Monitor (HYFED).

The GB levels in the chamber ($>0.005 \text{ mg/m}^3$) were continuously monitored with a phosphorus analyzer (HYFED, Model PH262, Columbia Scientific, Austin, TX). The analyzer output was recorded on a strip chart recorder, which showed the rise, equilibrium, and decay of the chamber vapor concentration during each experimental run. In addition, it gave a close approximation of the amount of GB (mg/m^3) in the chamber based on data (sorbent tube quantitation with HYFED response) from previous chamber runs.

2.6 Generation, Sampling, and Monitoring for Different Levels of GB Vapor.

2.6.1 GB Levels ($2\text{-}44 \text{ mg/m}^3$) High Range.

The spray atomizer was used to generate GB vapor concentrations $>1.0 \text{ mg/m}^3$. Five separate chamber runs were performed using the atomizer, with each run targeting a specific concentration between the range of $2.0\text{-}44 \text{ mg/m}^3$. Syringe drive settings ranged from $1.0\text{-}23 \text{ uL/min}$ with chamber flows of approximately $400\text{-}600 \text{ L/min}$ to achieve the vapor concentrations. Once the spray atomizer ($\sim 30 \text{ psi}$) was activated and the chamber had achieved equilibrium (t_{99}), vapor samples were drawn and collected onto solid sorbent tubes for subsequent GC-FID analysis. All sorbent tube samples were drawn intermittently at the rate of 0.1 L/min for $1\text{-}3 \text{ min}$, except for the 44 mg/m^3 concentration, which was drawn at $.025 \text{ L/min}$ for 1 min . In addition to the sorbent tube sampling, the chamber was continuously monitored with a phosphorus analyzer (HYFED) to visualize the chamber profile. Appendix D illustrates an experimental 1-hr chamber run monitored via the HYFED with sampling intervals indicated for the sorbent tubes.

2.6.2 GB Levels ($0.01\text{-}0.06 \text{ mg/m}^3$) Mid Range.

The saturator cell was used to generate GB vapor concentrations $<1.0 \text{ mg/m}^3$. Changes in concentration were made primarily through adjustments in water bath temperature and carrier flow through the cell (Table 2). Three separate (4 hr) chamber runs were conducted to evaluate the generator performance at concentrations of $0.01\text{-}0.06 \text{ mg/m}^3$ GB vapor. All sorbent tube samples were drawn at the rate of $0.2\text{-}0.3 \text{ L/min}$ and quantified by GC-FPD.

Table 2. Generator and Chamber Parameters for Medium-Level GB Vapor

Actual GB (mg/m^3)	Theoretical GB (mg/m^3)	N ₂ Flow Through Sat Cell (sccm)	Water Bath Temp (°C)	Chamber Flow (SLPM)
0.011	0.0113	1.6	15	1,365
0.04	0.041	4.8	18	1,388
0.062	0.063	7.7	18	1,446

2.6.3 GB Levels (0.0002-0.0035 mg/m³) Low Range.

The saturator cell was used to generate low GB vapor concentrations approaching the TLV-TWA of 0.0001 mg/m³.⁵ The primary method to attain these low concentrations was to significantly decrease the water bath temperature for the saturator cell as well as to decrease the carrier flow through the cell. A salt solution (23% sodium chloride dihydrate) was added to the water bath to depress its freezing point down to -20 °C, which in turn, significantly reduced the amount of vapor formation from the generator.

Three separate chamber runs (4-12 hr) were conducted to evaluate the generator performance at concentrations ranging from 0.0002-0.0035 mg/m³. Generator and chamber parameters used to achieve each concentration are listed in Table 3. All samples were drawn at the rate of 0.4 L/min for each concentration. Sample collection times varied from 5 min for the 0.0035 mg/m³ level up to 20-30 min for the 0.0002 mg/m³ levels.

Table 3. Generator and Chamber Parameters for Low-Level GB Vapor

Actual GB (mg/m ³)	Theoretical GB (mg/m ³)	N2 Flow Through Sat Cell (sccm)	Water Bath Temp (°C)	Chamber Flow (SLPM)
0.00024	0.00023	0.5	-18.1	1,492
0.0015	0.0016	0.5	5.8	1,492
0.0035	0.0033	1.0	5.8	1,492

3. RESULTS

The GB concentrations determined by sorbent tube GC analysis were plotted over time for each chamber run. Each chamber run consisted of a series of measurements taken for a specific concentration and run time. A combination of runs for each range (low, medium, high) were plotted together to examine the stability of the vapor generation systems at different concentrations over time. Figures 5 and 6 summarize the stability and range of the spray atomizer for five chamber runs at the high GB levels (2-44 mg/m³). Typically, this range was used to determine inhalation toxicity for lethality.

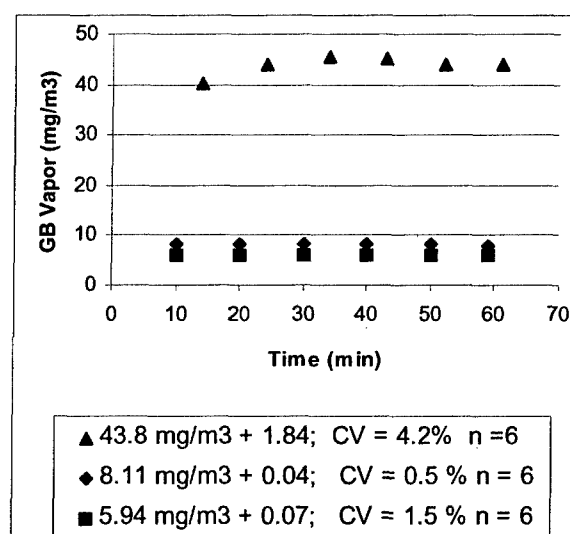


Figure 5. Spray Atomizer Generation of GB Vapor at the High Range (1 Hr)

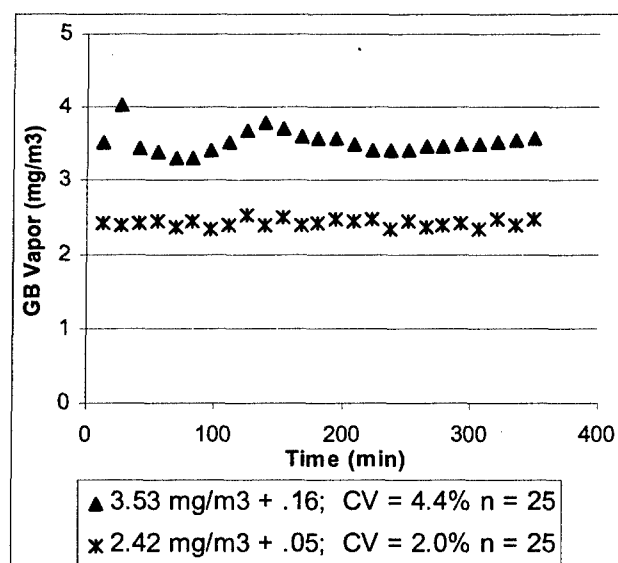


Figure 6. Stability of the Spray Atomizer for GB Vapor at the High Range (6 Hr)

Figure 7 summarizes the stability and range of the saturator cell for three chamber runs at the medium GB vapor levels (0.01-0.06 mg/m³). Typically, this range was used to access inhalation toxicity for sublethal effects (i.e., miosis).

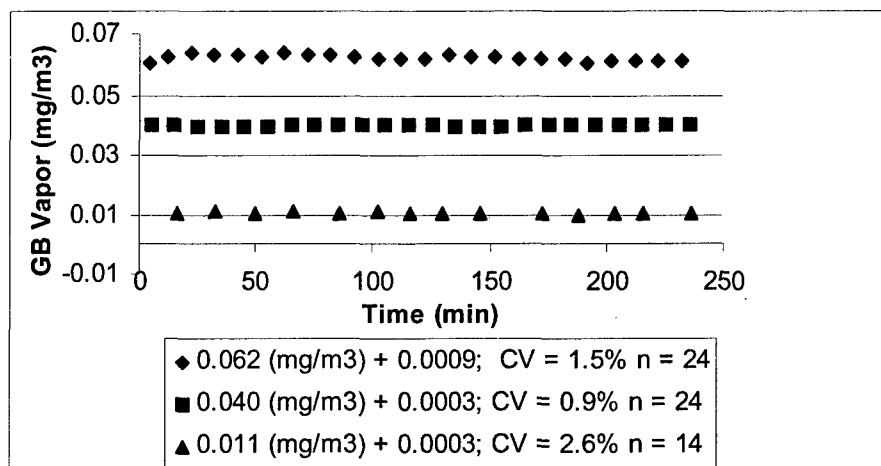


Figure 7. Stability of Saturator Cell to Generate GB Vapor at the Medium Range (4 Hr)

Figure 8 summarizes the stability and range of the saturator cell for three chamber runs at the low GB vapor levels (0.00025-0.0036 mg/m³). Typically, this range would be used to access inhalation toxicity for subclinical effects (i.e., EEG, blood, or tissue accumulation) and for extended miosis exposures.

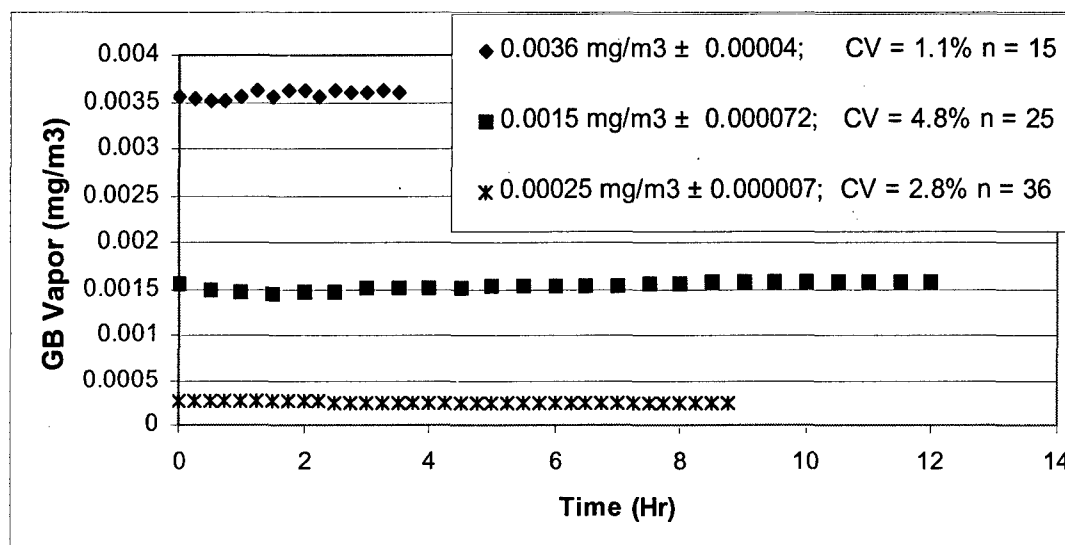


Figure 8. Stability of the Saturator Cell to Generate GB Vapor at the Low Range (4-12 Hr)

4. DISCUSSION

4.1 Vapor Generators.

Numerous vapor generation techniques have been developed for CW agents in toxicological studies. The type of vaporization technique used depended upon such factors as chemical volatility or vapor pressure, exposure concentration, and the size of the exposure system (inhalation chamber size and flowrate). Common generation methods described in the literature have included liquid sparging (carrier gas bubbled through a liquid reservoir), delta tube saturator, liquid-dispensing pumps (syringe pumps or metering pumps), gas dispersion bottles, metering devices, diffusion, and permeation devices.^{2,3} These techniques typically use a combination of temperature and carrier flow to establish and maintain a set vapor concentration.

Drawbacks to the liquid sparging and gas dispersion techniques are that they often require numerous control valves, large liquid reservoirs, and/or dilution systems prior to entry of vapor into the inhalation chamber. Simpler devices such as either a diffusion cell, delta tube saturator, or a permeation tube are good for small chambers (1-10 L) and small exposure applications (single animal, head-only, or nose-only exposures).¹ However, these devices do not generate enough vapor for whole body inhalation studies in a large chamber that may expose a group of animals or a single large animal.

For this study, the syringe pump-spray atomizer and saturator cell were considered ideal for generating stable GB vapor levels over a wide range of concentrations. Both systems were compact, easy to operate, and gave a good linear range for vapor generation.

4.1.1 Spray Atomizer.

The air atomizer generation technique had been used successfully for the vaporization of dilute GB in hexane, and therefore, was tested for the vaporization of neat GB.⁸ In this technique, the combination of pressure and orifice size were important parameters to ensure that vaporization was complete and that aerosols (identified by aerosol analyzers, or filter samples) were not formed in the chamber. One advantage of this system over heating methods was that it did not alter the characteristics of the chemical. In other words, once the spray atomization parameters were established to vaporize the agent in the chamber, the agent did not recondense back into an aerosol. Conversely, an agent that had been vaporized through heating had the potential to recondense back into an aerosol once it had hit the cooler temperature in the chamber.

4.1.2 Saturator Cell.

The saturator cell was originally used as a means to generate stable vapor concentrations to determine the vapor pressure of VX and DMMP at various carrier flows and temperatures.⁹ An extension of this capability was to use it as a continuous vapor source on a 750 L inhalation chamber. This generator worked similar to a sparging apparatus, however, the saturator cell contained a ceramic thimble that served as a wick to increase the surface area of the liquid GB. In addition, the nitrogen carrier gas made three passes over the surface of the ceramic

(liquid saturated) thimble as opposed to over or through the liquid. The small size of the cell required only 2-3 mL of liquid agent as opposed to a larger volume normally required for liquid reservoirs. Overall, the saturator cell was a simple and efficient means to generate GB vapor over a wide concentration range.

Vapor generation from the saturator cell followed the ideal gas law whereby

$$PV = nRT \quad (1)$$

where

P = Pressure (mm Hg)

R = Gas Constant

V = Volume (L)

T = Temperature (K)

n = g/mol

By rearranging the equation and substituting (L) for (V) we have

$$g/L = PMW / RT \quad (2)$$

The vapor pressure (P) of GB can be computed using Antoine's equation (eqs 3 and 4) and by applying the coefficients A = 8.579692, B = 2348.321 and C = 261.898 as determined by Penski¹⁰

$$\text{where} \quad \text{Log}_{10} P = A - B / (C + \text{Temp } ^\circ\text{C}) \quad (3)$$

$$\text{or} \quad P \text{ (mm Hg)} = 10 ^ { (A - B / (C + \text{Temp } ^\circ\text{C}))} \quad (4)$$

Thus, the concentration of GB vapor from the outlet of the saturator cell can be calculated from equation 5 as

$$\text{GB ug/min} = \frac{(P \text{ mm Hg}) (140.1 \text{ g/mol GB})}{62.4 \frac{(\text{mm Hg}) (\text{L})}{(\text{mole}) (\text{K})} (273.15 + ^\circ\text{C}) \text{ K}} \times (10^6 \text{ } \mu\text{g/g}) \times (\text{mL/min} \times .001 \text{ L/mL}) \quad (5)$$

where

$^\circ\text{C}$ = temperature of the water bath

mL/min = carrier flow through the saturator cell

The nominal chamber concentration can be calculated by dividing the GB concentration from the saturator cell (microgram/minute) by the chamber flow (liter/minute) to obtain GB microgram/liter. However, other factors such as the negative pressure exerted by the chamber flow on the saturator cell, as well as the deposition of GB vapor on the chamber walls, affected the final vapor concentration.

4.2 Vapor Stability in the Chamber.

The spray atomizer generated the high GB vapor concentrations at a range that could easily achieve 1-50 mg/m³. Out of five separate chamber runs (range 2-44 mg/m³) the

variation of GB vapor concentration in the chamber was <5% over a 1-6 hr period. Specifically, the variance was $\leq 2\%$ for three of the runs and 4% for the other two runs.

The saturator cell generated the medium and low GB vapor concentrations at a range of approximately 0.0002-0.1 mg/m³. The medium concentrations (.01-.06 mg/m³), were generated under parameters that were easy to achieve (15-16 °C, 1-8 mL/min) and represented the range for GB miosis in rats performed by Mioduszewski et al.¹¹ Variations for three separate chamber runs were within 2-3% over a 4-hr period.

The low GB concentrations (.0002-0.04 mg/m³) typically represented the toxicity range for subclinical signs or for miosis (extended exposure). For instance, Van Helden et al., conducted low-level acute exposures for 5 hr to examine the lowest observable effects of GB exposure in guinea pigs and marmosets.¹² Miosis was observed at exposure concentrations ranging from 0.0075-15 mg/m³ for guinea pigs and .0073-138 mg/m³ for marmosets.¹² These concentrations were well within the range for the low levels tested in this study. Variance for the 3 chamber runs in this study was within 1-5%. Chamber conditioning time was significantly greater at this level than the previous higher concentrations, and the generator temperature had to be set significantly lower than for the mid-range concentrations.

4.3 GB Vapor Sampling and Analysis System.

A verification of the sampling and analysis system for GB vapor was conducted by Muse et al.,¹³ prior to this study. High GB vapor concentrations (1-40 mg/m³) were generated in a 750 L inhalation chamber by the spray atomization system. Numerous GB vapor samples from the chamber were independently drawn and analyzed with solvent bubblers, as well as the automated sorbent tube system (Dynatherm) described in this study. A statistical comparison (t-test of the means) showed no significant difference between the two sampling methods. This comparison was conducted at the high concentration level to verify the performance of the automated sorbent tube sampling system for use at medium and low GB levels, where bubbler sampling and analysis would be impractical.

5. CONCLUSIONS

This paper describes techniques used for the generation, sampling and analysis of sarin (GB) vapor at various toxicological significant levels. The spray atomization system was an effective generator for the high (lethal range) GB vapor concentrations (1-50 mg/m³). The saturator cell generator was most effective at sub-lethal concentrations and demonstrated an effective range up to three orders of magnitude (0.00025-0.1 mg/m³) GB. Both generators produced stable vapor concentrations for an extended period of time with variations ranging from 1-5%. In addition, the sampling and analysis system was a rapid and sensitive method for performing low-level GB vapor studies. With adaptations, these techniques should be useful for testing less volatile agents such as GF and VX.

LITERATURE CITED

1. McClellan, R.O.; Henderson, R.F. *Generation and Characterization of Gases and Vapors*; Hemisphere Publishing Corporation: New York, 1989.
2. Vieira, B.M.; Freitas, M.M.; Cardoso, L.M.N.; Neto, G. The Evaluation of a System for Generation of Atmospheres with Standard Concentration of Vapors for Use in Acute Inhalation Toxicity Studies. *J. Environ. Sci. Health*, **1991**, A26(4), pp 455-469.
3. Tevault, D.E.; Ong, K.Y.; Wasserman, M.B.; Ahearn, W. *Vapor Generation Methods for Chemical Warfare Agents*; ECBC-TR-148; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2001; UNCLASSIFIED Report (AD-A389 503).
4. Romano, J.A.; McDonough, J.H.; Sheridan, R.; Sidell, F.R. *Health Effects of Low-Level Exposure to Nerve Agents*; CRC Press: Boca Raton, 2001.
5. Mioduszewski, R.J.; Reutter, S.A.; Miller, L.L.; Olajos, E.J.; Thomson S.A. *Evaluation of Airborne Exposure Limits for G-Agents: Occupational and General Population Exposure Criteria*; ERDEC-TR-489; U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1998; UNCLASSIFIED Report (AD-A345 618).
6. Brickhouse, M.D.; Rees, M.S.; O'Connor, R.J.; Durst, H.D. *Nuclear Magnetic Resonance (NMR) Analysis of Chemical Agents and Reaction Masses Produced by their Chemical Neutralization*; ERDEC-TR-449; U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1997; UNCLASSIFIED Report (AD-A339 308).
7. *Chemical Agent Data Sheets, Vol. I*; EO-SR 74001; Edgewood Arsenal: Aberdeen Proving Ground, MD, 1974, UNCLASSIFIED Report (AD-B028 222).
8. Muse, W.T.; Anthony, J.S.; Thomson, S.A.; Crouse, C.L.; Crouse, L.C.B. *Acute Inhalation Toxicity of RDTE Dilute GB/Hexane in Rats*; ECBC-TR-147; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2001, UNCLASSIFIED Report (AD-A371 297).
9. Buchanan, J.H.; Buettner, L.C.; Butrow, A.B.; Tevault, D.E. *Vapor Pressure of VX*; ECBC-TR-068; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 1998; UNCLASSIFIED (AD-A371 297)
10. Penski, E.C. *The Properties of 2-Propyl Mehtylfluorophosphonate (GB) I. Vapor Pressure Data Review and Analysis*; ERDEC-TR-166; U.S. Army Edgewood Research, Development and Engineering Center: Aberdeen Proving Ground, MD, 1994, UNCLASSIFIED Report (AD-B187 225).

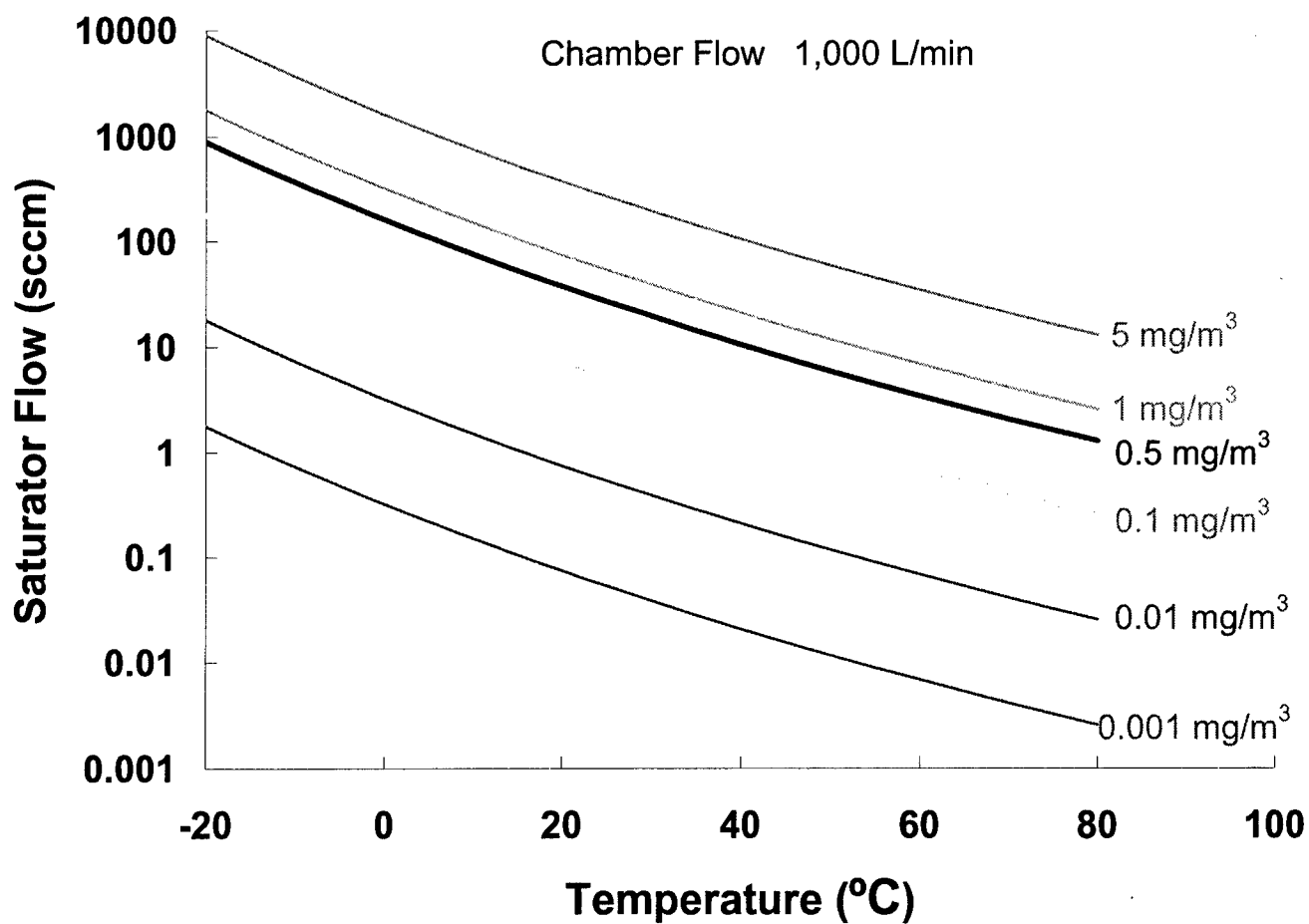
11. Mioduszewski, R.J.; Manthei, J.H.; Way, R.A.; Burnett, D.C.; Gavilola, B.P.; Muse, W.T.; Thomson, S.A.; Sommerville, D.R.; Crosier, R.B.; Scotto, J.A.; McCaskey, D.A.; Crouse, C.L.; Matson, K.L. *Low-Level Sarin Vapor Exposure in Rats: Effect of Exposure Concentration and Duration on Pupil Size*; ECBC-TR-235; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2002; UNCLASSIFIED Report (AD-A402 869).

12. Van Helden, H.P.M.; Langenberg, J.P.; Benschop H.P. *Low Level Exposure to GB Vapor in Air: Diagnosis/Dosimetry, Lowest Observable Effect Level and Performance Incapacitation*; In Final Report Cooperative Agreement DAMD17-97-1-7360; TNO Prins Maurits Laboratory: Rijswijk, The Netherlands; NTIS (AD-A 394639/XAB 97-2000).

13. Muse, W.T.; Anthony, J.S.; Buettner, L.C.; Durst, H.D.; Mioduszewski, R.J.; Thomson, S.A.; Crouse, C.L.; Crouse, L.C.B. *Comparison of Bubbler Versus Sorbent Tube Sampling For the Analysis of GB (Sarin) Vapor for Inhalation Toxicology*; ECBC-TR-202; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2002; UNCLASSIFIED Report (AD-A400-830).

APPENDIX A

GB VAPOR CONCENTRATIONS FROM A SATURATOR CELL AT VARIOUS TEMPERATURE AND CARRIER FLOWS



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APPENDIX B

GC PARAMETERS FOR GB ANALYSIS

GC/FPD Operation for Dynatherm

Gas chromatograph	Hewlett Packard 6890
Capillary column	DB-5, 30 m x 0.32 mm i.d., x 1.0 mm film thickness
Column flow (He)	Velocity = 64 cm/sec; Head pres = 20.0 psi initial, ramp to 40 psi – 1 min
Detector flow (FPD)	100 mL/min (air); 75 mL/min (H ₂); 15 mL/min (He)
Detector temp (FPD)	200 °C
Col temperature program	75 °C (hold 0.5 min) to 130 °C @ 20 °C/min (run time: 4 min)

GC/FID Operation for Dynatherm

Same Chromatographic Parameters as above except:

Detector flow (FID)	450 mL/min (air); 40 mL/min (H ₂); 45 mL/min (He)
Detector temp (FID)	250 °C

Instrumental Parameters for Thermal Desorption

Model: Dynatherm (ACEM 900)

Temperature/Flow Program:

Tube Idle	40 °C	Tube Dry	1 min
Transfer Line	175 °C	Tube Heat	2 min
Tube Desorb	260 °C	Tube Cool	0 min
Trap Desorb	300 °C	Trap Heat	2 min
Valve Temp	150 °C		
Purge Flow	5 mL/min (He)		
Solid Sorbent	Tenax TA/Haysep (10 cm x 6 mm o.d.)		

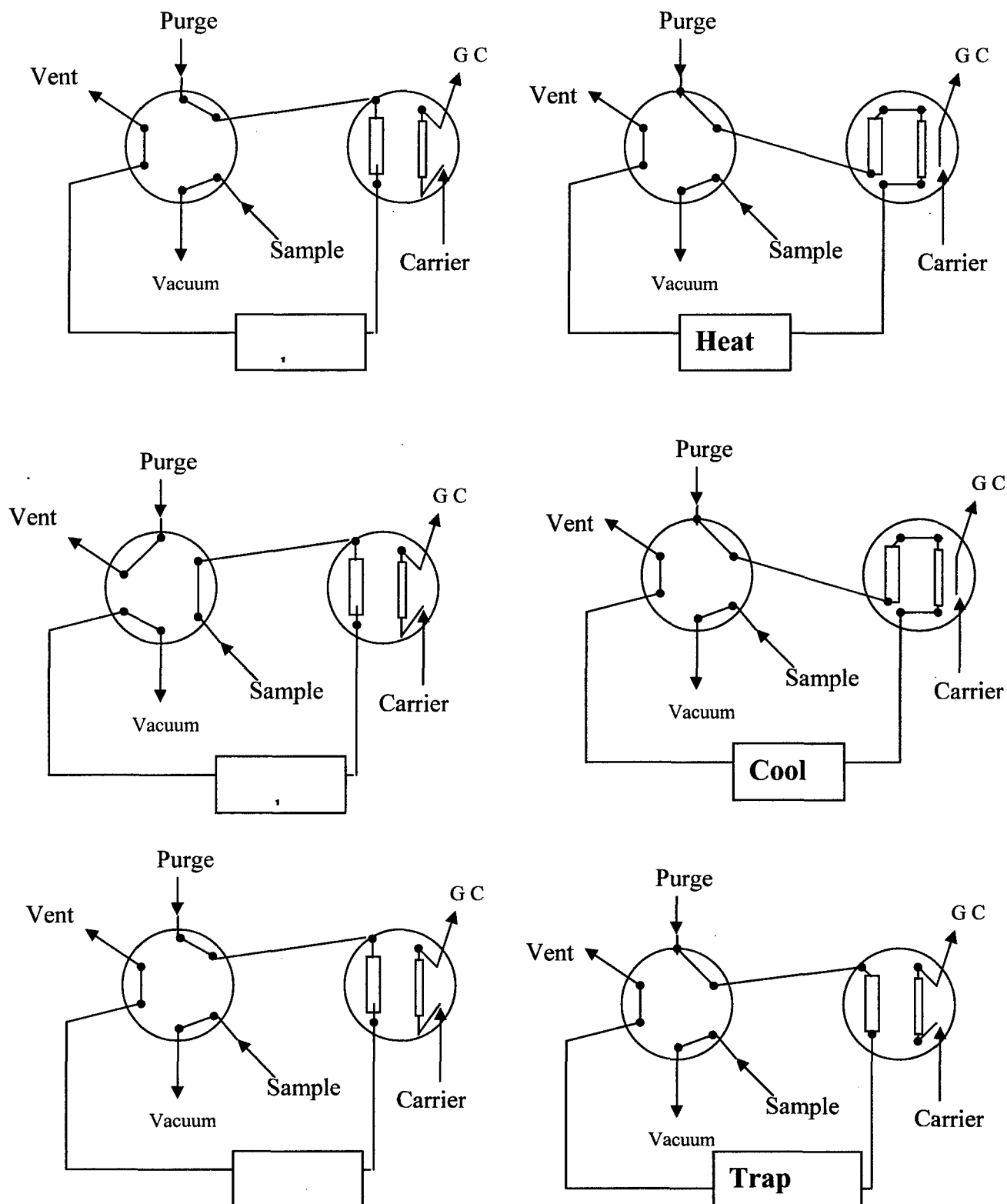
Sample Time:

External Sample	External Standard Calibration through sample line	5-7 min
	Chamber Sample	1-5 min

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APPENDIX C

VALVING POSITIONS FOR SAMPLE SWITCHING VALVE AND DYNATHERM



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APPENDIX D

HYFED PROFILE FOR GB VAPOR IN AN INHALATION CHAMBER WITH CONCURRENT DYNATHERM SAMPLES

Dynatherm Samples (1 - 5)

